



TITLE:

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CITATION:

So, Kanako ...[et al]. Involvement of TRPM2 in a wide range of inflammatory and neuropathic pain mouse models.. Journal of pharmacological sciences 2015, 127(3): 237-243

ISSUE DATE:

2015-03

URL:

<http://hdl.handle.net/2433/201978>

RIGHT:

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Journal of Pharmacological Sciences

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Full paper

Involvement of TRPM2 in a wide range of inflammatory and neuropathic pain mouse models



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ARTICLE INFO

Article history:

Received 5 August 2014

Received in revised form

16 October 2014

Accepted 23 October 2014

Available online 18 February 2015

Keywords:

TRPM2

Inflammatory pain

Neuropathic pain

Pain models

Knockout mice

ABSTRACT

Recent evidence suggests a role of transient receptor potential melastatin 2 (TRPM2) in immune and inflammatory responses. We previously reported that TRPM2 deficiency attenuated inflammatory and neuropathic pain in some pain mouse models, including formalin- or carrageenan-induced inflammatory pain, and peripheral nerve injury-induced neuropathic pain models, while it had no effect on the basal mechanical and thermal nociceptive sensitivities. In this study, we further explored the involvement of TRPM2 in various pain models using TRPM2-knockout mice. There were no differences in the chemonociceptive behaviors evoked by intraplantar injection of capsaicin or hydrogen peroxide between wildtype and TRPM2-knockout mice, while acetic acid-induced writhing behavior was significantly attenuated in TRPM2-knockout mice. In the postoperative incisional pain model, no difference in mechanical allodynia was observed between the two genotypes. By contrast, mechanical allodynia in the monosodium iodoacetate-induced osteoarthritis pain model and the experimental autoimmune encephalomyelitis model were significantly attenuated in TRPM2-knockout mice. Furthermore, mechanical allodynia in paclitaxel-induced peripheral neuropathy and streptozotocin-induced painful diabetic neuropathy models were significantly attenuated in TRPM2-knockout mice. Taken together, these results suggest that TRPM2 plays roles in a wide range of pathological pain models based on peripheral and central neuroinflammation, rather than physiological nociceptive pain.

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1. Introduction

Pathological pain is mediated through pathologically enhanced pain pathways induced by a variety of causes, such as inflammation, arthritis, surgical incision, peripheral/central nerve injury, demyelination, diabetes, and some classes of chemotherapeutic agents. Several lines of evidence suggest that neuroinflammation mediated by the interaction between immune cells and nociceptive neurons plays a key role in pathological pain (1,2). Circulating immune cells

infiltrate in response to peripheral tissue damage, inflammation, or nerve injury. Pronociceptive inflammatory mediators such as proinflammatory cytokines, chemokines, and reactive oxygen species (ROS) released from the infiltrated immune cells can increase the sensitivity of nociceptive primary sensory neurons, *i.e.* peripheral sensitization. In addition, the prolonged or intense hyperexcitability of peripheral nociceptive neurons can trigger hyperexcitability of the nociceptive dorsal horn neurons in the spinal cord, *i.e.* central sensitization. Accumulating evidence suggests that the activation of spinal glial cells, such as microglia and astrocytes, contribute to the generation of central sensitization thorough the production of pronociceptive inflammatory mediators (3).

Transient receptor potential melastatin 2 (TRPM2) channel is expressed abundantly in immune cells, including monocytes/macrophages, neutrophils, T-lymphocytes, and microglia (4–6), and acts

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Peer review under responsibility of Japanese Pharmacological Society.

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as a sensor for ROS (7–9). A growing body of evidence suggests a role for TRPM2 in immune and inflammatory responses (6,10–14). Recently, we reported the involvement of TRPM2 expressed on macrophages and microglia in pathological pain (15,16). We showed that painful behaviors in inflammatory pain models (such as the second phase of the formalin test, and the carrageenan-induced inflammatory pain model) and neuropathic pain models induced by peripheral nerve injury (such as partial sciatic nerve ligation and spinal nerve transection) are attenuated in TRPM2-knockout (KO) mice. By contrast, the basal thermal (Hargreaves test and hot plate test), mechanical (von Frey filament test), and chemical (first phase of the formalin test) nociceptive sensitivities were unaffected in TRPM2-KO mice. These findings suggest that TRPM2 could be a promising drug target for the treatment of pathological pain. However, pathological pain can arise from not only tissue inflammation and peripheral nerve injury, but also from other causes. Recent studies established a variety of rodent models of pathological pain that show more clinically relevant pain states (17). In this study, to explore the types of pain mediated via TRPM2, we further examined the effects of TRPM2 deficiency in various mouse pain models, including capsaicin or hydrogen peroxide (H_2O_2)-evoked chemical nociceptive pain, acetic acid-induced writhing behaviors, osteoarthritis pain, postoperative pain, chronic pain in multiple sclerosis models, painful diabetic neuropathy, and chemotherapy-induced peripheral neuropathy in mice.

2. Methods

2.1. Animals

This study was carried out in strict accordance with the recommendations of the Guiding Principles for the Care and Use of Animals of The Japanese Pharmacological Society. The protocol was approved by the Kyoto University Animal Research Committee (Permit Number: 2012–24 and 2013–24). All efforts were made to minimize the number of animals used, and limit experimentation to only what was necessary to produce, as previously reported. Male or female mice aged 7–12 weeks were used in this study. TRPM2-KO mice were generated as previously reported (12). The TRPM2-KO mouse line was backcrossed with C57BL/6J mice for seven to ten generations to eliminate any background effects on the phenotypes. The mice were kept at a constant ambient temperature of $24 \pm 1^\circ\text{C}$ under a 12 h light/dark cycle with free access to food and water.

2.2. von Frey filament test

Mechanical sensitivity was assessed by the up-down method using calibrated von Frey filaments as previously described, with slight modifications (18,19). Mice were acclimatized on a metal mesh floor in small cylinders for 2 h. The mechanical sensitivity was evaluated using a set of seven calibrated von Frey filaments (0.008, 0.02, 0.04, 0.07, 0.16, 0.4, and 1.0 g; Stoelting) that were applied to the plantar surface of the hind paw until the filament bent slightly for a few seconds. The 0.16 g filament was always the first stimulus. When there was a positive response, such as flicking or lifting, the next lower filament was applied, and when there was no response, the next higher filament was used. The 50% paw withdrawal threshold value was calculated from five consecutive responses (18,20).

2.3. Chemical nociceptive behavior to the intraplantar injection of capsaicin or H_2O_2

Chemical nociceptive behavior to the intraplantar injection of capsaicin or H_2O_2 was assessed as previously described, with slight

modifications (21,22). Capsaicin (80 $\mu\text{g}/\text{ml}$; Nacalai Tesque, Kyoto, Japan) and H_2O_2 (0.3%; Wako Pure Chemical Industries, Ltd., Osaka, Japan) were prepared in corn oil and sterile saline (0.9%), respectively. Male WT and TRPM2-KO mice were individually acclimatized to an acrylic observation chamber 25 cm in diameter and 30 cm in height for approximately 1 h before the injection. 20 μL of capsaicin (1.6 $\mu\text{g}/\text{paw}$) or 0.3% H_2O_2 was injected in the plantar surface of the right hind paw, using a Hamilton syringe with a 30-gauge needle. The mouse was then put in the chamber and the period of observation started. Vehicle control animals, receiving an intraplantar injection of corn oil or saline, were also observed under identical conditions. Because both capsaicin- and H_2O_2 -evoked nociceptive behaviors appear immediately, and decay within 5 min (21,22), the total time spent licking and biting the injected paw was recorded for 5 min after the injection.

2.4. Acetic acid-induced writhing test

WT and TRPM2-KO mice were acclimatized in individual observation cages for 1 h. The mice were intraperitoneally injected with a 0.9% acetic acid solution (10 ml/kg), and then returned to their cages. The number of typical writhing behaviors, characterized by the contraction of the abdominal musculature followed by extension of the hind limbs, was counted for 30 min.

2.5. Postoperative incisional pain model

For the postoperative pain model, the surgery was performed as previously described (23), and adapted to mice (24). Briefly, male WT and TRPM2-KO mice were anesthetized with isoflurane. After sterile preparation of the right hind paw, a 5 mm longitudinal incision was made through the skin and fascia of the plantar surface using a No. 11 scalpel blade. The incision started 2 mm from the proximal edge of the heel and extended toward the toes. The plantaris muscle was elevated with forceps, leaving the muscle origin and insertion intact. The skin was closed with one single 7–0 surgical silk suture. Mechanical sensitivity was assessed before and 6 h, 1, 3, 5, 7, 10 and 14 days after the incision, as described above.

2.6. Monosodium iodoacetate (MIA)-induced osteoarthritis pain model

Osteoarthritis was induced by a single intra-articular injection of MIA (Sigma–Aldrich, St. Louis, MO) into the knee, following a previously described protocol, with slight modifications (25). Briefly, male WT and TRPM2-KO mice were anesthetized with isoflurane, and the knee joints were shaved and flexed at a 90° angle. 10 μL of 10 mg/ml MIA in sterile saline (0.9%) was injected through the infra-patellar ligament into the joint space of the right (ipsilateral) knee using a 30-gauge needle. Mice in which the 50% paw withdrawal threshold was decreased under 0.4 g at 21 days after the MIA administration were used. Mechanical sensitivity was assessed before and 1, 3, 7, 10, 14 and 21 days after the intra-articular injection, as described above.

2.7. Induction of the experimental autoimmune encephalomyelitis (EAE) model and assessment of the clinical severity of the EAE

For the induction of the EAE model, the procedures were performed as previously described (26). Female WT and TRPM2-KO mice were subcutaneously (s.c.) immunized in both flanks with the MOG_{35–55} peptide (20 or 100 μg per animal; Operon Biotechnologies, Inc., Tokyo, Japan) in 200 μL of an emulsion consisting of a 1:1 (volume/volume) mixture of physiological saline and complete Freund's adjuvant (CFA), the latter of which contained

Mycobacterium tuberculosis H37RA at 5 mg/mL (Becton Dickinson & Co., Franklin Lakes, NJ). An i.p. injection of 200 ng pertussis toxin (List Biological Laboratories, Inc., Campbell, CA) was administered at the time of the induction and again 48 h later. Control mice were treated with CFA and pertussis toxin alone. For the assessment of the clinical severity of the EAE, the clinical scores of EAE were graded according to the following scale: Score 0, normal mouse; Score 1, tail paralysis; Score 2, mild hind limb weakness; Score 3, moderate to severe hind limb paralysis and/or mild forelimb weakness; Score 4, complete hind limb paralysis and/or moderate to severe forelimb weakness; Score 5, quadriplegia or moribund state; Score 6, death. Mice were monitored daily for clinical signs of EAE. Mechanical sensitivity was assessed before and 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 22, 25 and 27 days after the treatment, as described above.

2.8. Paclitaxel-induced neuropathic pain model

Paclitaxel (Sigma–Aldrich) was dissolved in cremophor:ethanol:saline (5:5:90, v/v/v). Male WT and TRPM2-KO mice were injected with paclitaxel (4 mg/kg; i.p.) once daily on day 0, 3, 5, and 7, as previously described, with slight modifications (27). Mice injected with the ethanol:cremophor:saline vehicle were used as control mice. Mechanical sensitivity was assessed before and 4, 7, 14 and 21 days after the injection, as described above.

2.9. Streptozotocin (STZ)-induced diabetic neuropathic pain model

Diabetes was induced by tail vein injection of STZ (Sigma–Aldrich), dissolved in sterile saline (0.9%), at a dose of 75 mg/kg into male WT and TRPM2-KO mice. Blood was sampled from the tail vein, and the glucose level was measured by using the One-Touch Ultra Blood Glucose Monitoring System (Johnson & Johnson, New

Brunswick, NJ). Mice were deemed diabetic when the blood glucose concentration exceeded 200 mg/dL. Mechanical sensitivity was assessed before and 7 and 14 days after the injection, as described above.

2.10. Statistical analysis

Data were expressed as means \pm S.E.M. Statistical analyses of the 50% paw withdrawal thresholds were performed using the Mann–Whitney U-test at the individual time points. The EAE scores and blood glucose levels were analyzed by a two-way analysis of variance (ANOVA), followed by the Bonferroni's *post hoc* comparison test. To analyze other behavioral responses, we used the Student's *t*-test for comparisons between two mean values. Differences with $P < 0.05$ were considered significant.

3. Results

3.1. Nociceptive responses to chemical stimuli

Nociceptive responses to acute chemical stimuli were compared between wildtype (WT) and TRPM2-KO mice. Following an intraplantar (i.pl.) injection of capsaicin, licking and biting were observed in both WT and TRPM2-KO mice, but no nociceptive behaviors were observed after an i.pl. injection of the vehicle. No significant difference was observed between WT and TRPM2-KO mice with respect to the duration of capsaicin-evoked nociceptive behaviors (Fig. 1A). Similarly, there was no significant difference in the duration of licking and biting evoked by an i.pl. injection of H_2O_2 between WT and TRPM2-KO mice (Fig. 1B).

3.2. Acetic acid-induced writhing test

An intraperitoneal (i.p.) injection of acetic acid into WT mice induced writhing behavior, which peaked 5–10 min after the injection. TRPM2-KO mice exhibited significantly fewer writhing behaviors than the WT mice (Fig. 2).

3.3. Postoperative incisional pain model

A surgical incision in the plantar aspect of the hind paw decreased the 50% paw withdrawal thresholds of the ipsilateral hind paw in both WT and TRPM2-KO mice. The withdrawal thresholds peaked at 6 h, and lasted for at least 14 days after the incision. There was no significant difference between WT and

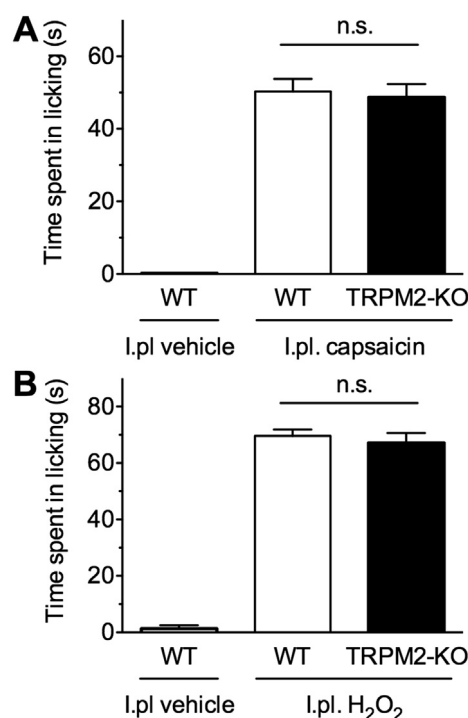


Fig. 1. Effect of TRPM2 deficiency on capsaicin or H_2O_2 -evoked chemical nociceptive behaviors. WT and TRPM2-KO mice were injected intraplantarly (i.pl.) with (A) vehicle or capsaicin (1.6 μ g/paw), (B) saline or 0.3% H_2O_2 solution. The duration of licking and biting behaviors to the injected paw was measured for 5 min following the injection $n = 4$ –6.

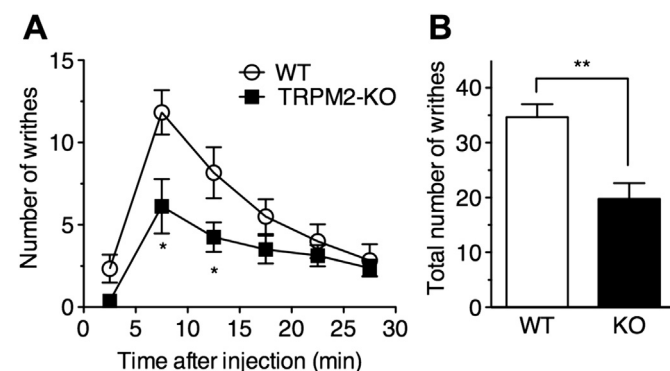


Fig. 2. Effect of TRPM2 deficiency on acetic acid-induced writhing behaviors. WT ($n = 6$) and TRPM2-KO mice ($n = 8$) were injected intraperitoneally (i.p.) with 0.9% acetic acid solution (10 ml/kg). The number of typical writhing behaviors was counted every 5-min over 30 min (A). * $P < 0.05$, compared with WT mice. (B) Total number of writhing behaviors for 30 min ** $P < 0.01$.

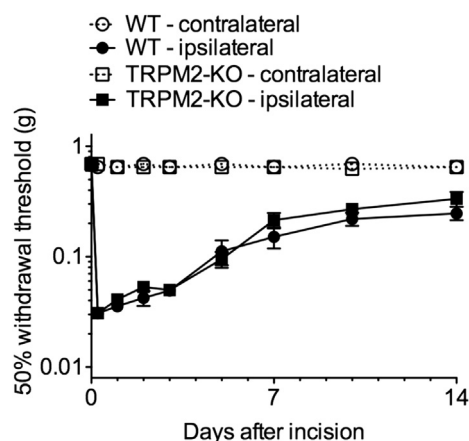


Fig. 3. Effect of TRPM2 deficiency on mechanical allodynia in the postoperative incisional pain model. The plantar surface of WT and TRPM2-KO mice were incised, as described in the Materials and Methods. The 50% paw withdrawal thresholds in the ipsilateral and contralateral paws were determined 6 h, 1, 2, 3, 5, 7, 10 and 14 days after the incision $n = 7$.

TRPM2-KO mice at any of the time points. The 50% paw withdrawal thresholds of the contralateral hind paw were unaffected by the incision (Fig. 3).

3.4. MIA-induced osteoarthritis pain model

An intra-articular injection of MIA, but not of saline, into the knee decreased the 50% paw withdrawal threshold of the ipsilateral hind paw. The number of MIA-induced osteoarthritis-developed mice, in which the 50% paw withdrawal threshold was lower than 0.4 g, was six out of nine for WT mice and five out of ten for TRPM2-KO mice, respectively. There was no significant difference in the incidence rate of osteoarthritis between WT and TRPM2-KO mice. However, the decrease in 50% paw withdrawal threshold in TRPM2-KO mice was significantly attenuated compared with that in WT mice (Fig. 4).

3.5. Clinical signs and mechanical allodynia in EAE model

When WT and TRPM2-KO mice were treated with CFA and pertussis toxin alone, in the absence of myelin oligodendrocyte glycoprotein (MOG)_{35–55} peptide, the clinical signs of EAE were not

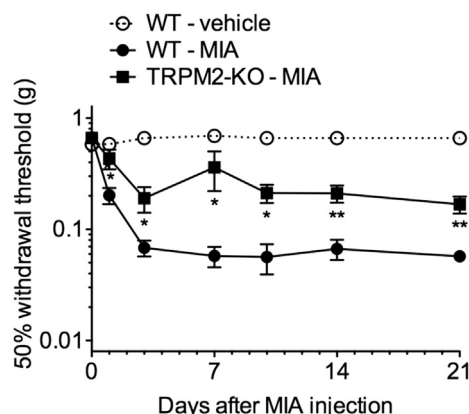


Fig. 4. Effect of TRPM2 deficiency on mechanical allodynia in the MIA-induced osteoarthritis pain model. Vehicle or MIA was injected intra-articularly into the knee of WT and TRPM2-KO mice. The 50% paw withdrawal thresholds in the ipsilateral paws were determined. $n = 5–6$. * $P < 0.05$, ** $P < 0.01$ vs ipsilateral-WT mice.

observed. When WT mice were immunized with 100 μ g MOG_{35–55} peptide, the mice began to develop clinical signs between 11 and 12 days post-immunization. The clinical score of the EAE in TRPM2-KO mice was significantly attenuated compared with that in WT mice (Fig. 5A). However, because EAE induced by 100 μ g MOG_{35–55} peptide showed severe clinical signs including paralysis, it was impossible to precisely measure the 50% paw withdrawal threshold. Therefore, mice were immunized with a lower dose of MOG_{35–55} peptide to induce weak EAE severity. When WT mice were immunized with 20 μ g MOG_{35–55} peptide, weak clinical signs were observed between 15 and 28 days, which did not include paralysis. The clinical score of the EAE in TRPM2-KO mice was attenuated compared with that in WT mice (Fig. 5B). The 50% paw withdrawal threshold began to decrease at day 1, and reached a plateau by 3 days post-immunization. In TRPM2-KO mice, the decrease in 50% paw withdrawal threshold was significantly

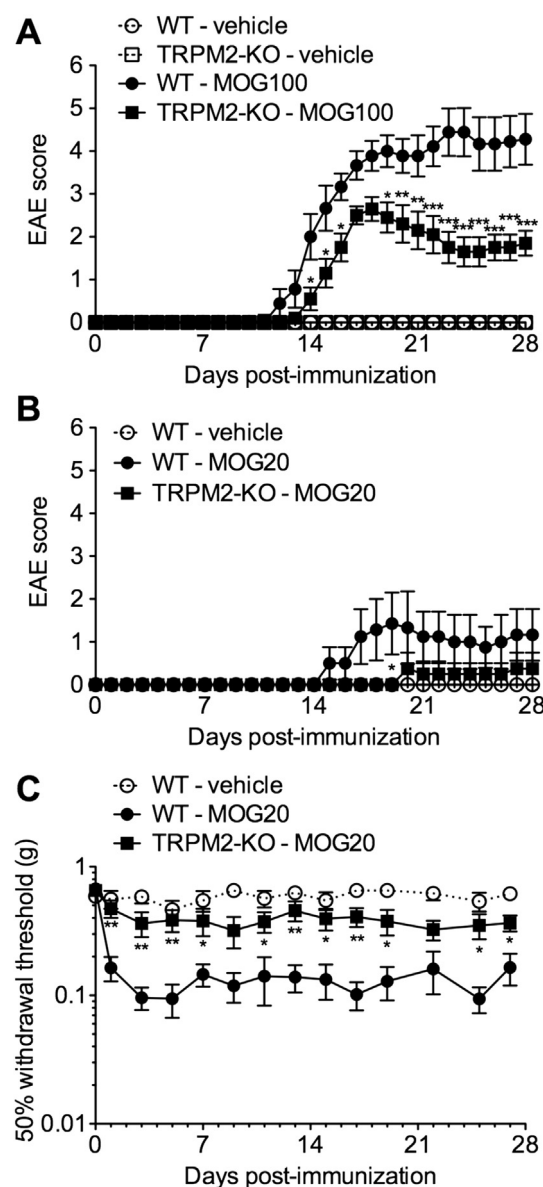


Fig. 5. Effect of TRPM2 deficiency on clinical signs and mechanical allodynia in the EAE model. Female WT and TRPM2-KO mice were immunized with vehicle, or with 100 μ g (A; MOG100) or 20 μ g (B, C; MOG20) MOG_{35–55} peptide. (A, B) Clinical signs of EAE were scored daily over 28 days after post-immunization. (C) The 50% paw withdrawal threshold was determined. $n = 4–10$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs WT mice.

attenuated compared with WT mice. On the other hand, vehicle treatment without MOG_{35–55} peptide did not change the 50% paw withdrawal threshold (Fig. 5C).

3.6. Paclitaxel-induced neuropathic pain model

When WT mice were repeatedly treated i.p. with paclitaxel (4 mg/kg; 4 times), the 50% paw withdrawal threshold was gradually decreased 4, 7, 14 and 21 days after the first injection, and remained unchanged even after repeated injection of vehicle using the same schedule. In TRPM2-KO mice, the decrease in 50% paw withdrawal threshold was significantly attenuated compared with that in WT mice (Fig. 6).

3.7. STZ-induced diabetic neuropathic pain model

Fourteen days after the STZ-treatment, the blood glucose level significantly increased compared with that of the vehicle-treated control mice. There was no significant difference in the blood glucose levels between WT and TRPM2-KO mice after vehicle- or STZ-treatment, although TRPM2-KO mice showed slightly higher blood glucose levels than WT mice (Fig. 7A). In WT mice treated with STZ, the 50% paw withdrawal threshold decreased over 7 days, and recovered 14 days after the treatment, but remained unchanged in the vehicle-treated mice. In TRPM2-KO mice, the decrease in 50% paw withdrawal threshold 7 days after the STZ-treatment was significantly attenuated compared with that in WT mice (Fig. 7B).

4. Discussion

In the present study, we showed that TRPM2 deficiency attenuated painful behavior in various pathological pain models including acetic acid-induced writhing behavior, MIA-induced osteoarthritis pain, chronic pain in the EAE model, paclitaxel-induced peripheral neuropathy, and STZ-induced painful diabetic neuropathy. By contrast, TRPM2 deficiency had no effect on capsaicin- or H₂O₂-evoked chemical nociceptive pain or on mechanical allodynia in the postoperative incisional pain model. These results suggest that TRPM2 plays key roles in a wide range of pathological inflammatory and neuropathic pain, but not in nociceptive pain.

Nociceptive pain is defined as “pain arising from activation of nociceptors” (28). Nociceptive pain is generated when thermal,

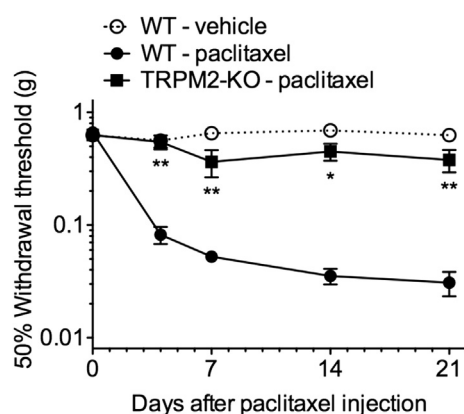


Fig. 6. Effect of TRPM2 deficiency on mechanical allodynia in the paclitaxel-induced peripheral neuropathic pain model. WT and TRPM2-KO mice were injected intraperitoneally with vehicle or paclitaxel (4 mg/kg; 4 times). The 50% paw withdrawal thresholds were determined. $n = 4–5$. * $P < 0.05$, ** $P < 0.01$ vs WT mice.

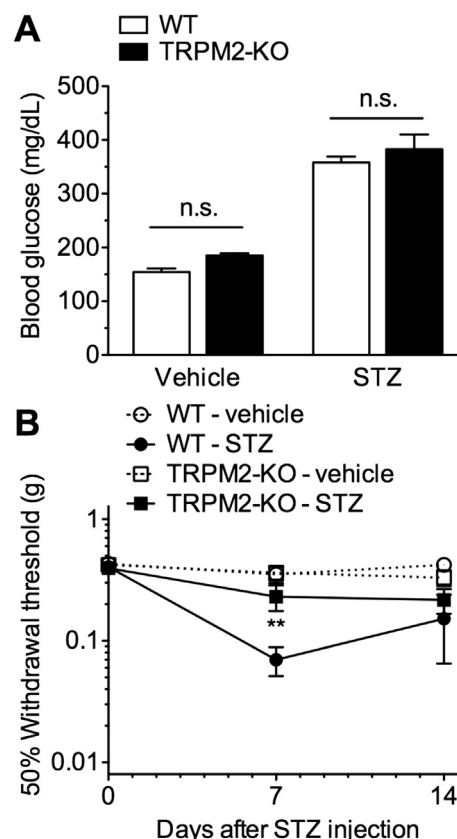


Fig. 7. Effect of TRPM2 deficiency on blood glucose level and mechanical allodynia in the STZ-induced peripheral neuropathic pain model. WT and TRPM2-KO mice were injected intravenously (i.v.) with vehicle or STZ (75 mg/kg). (A) Blood glucose levels were measured 14 days after STZ injection. (B) The 50% paw withdrawal thresholds were determined. $n = 5$. ** $P < 0.01$ vs STZ-WT mice.

mechanical, or chemical noxious stimuli activate nociceptors located on the peripheral terminals of primary sensory neurons. Tissue injury, inflammation or infection produces various pronociceptive mediators, which activate nociceptors to elicit nociceptive pain (29). Capsaicin and H₂O₂-evoked nociceptive behaviors represent the acute chemonociceptive pain in responses to stimulation of TRPV1 and TRPA1 (22,30). Although recent studies reported that functional TRPM2 is also expressed in the dorsal root ganglion neurons (31), the present results, taken together with our previous findings (15), suggest that TRPM2 expressed on sensory neurons plays no role, at least, in exogenous ROS-evoked acute nociceptive pain. By contrast, TRPM2 deficiency attenuated the acetic acid-induced writhing behavior, which is recognized as an acute inflammatory pain model. The acetic acid-induced writhing response is mediated by the rapid infiltration of neutrophils into the peritoneal cavity. Neutrophil depletion by the treatment with anti-Ly6G antibody attenuates the acetic acid-induced writhing response (32). We reported that TRPM2 deficiency reduces the infiltration of neutrophils into the inflamed sites, resulting inhibition of mechanical allodynia and thermal hyperalgesia in carrageenan-induced inflammatory pain model (15). Taken together, these findings suggest that TRPM2 is involved in acetic acid induced inflammatory pain, but not in acute chemonociceptive pain signaling.

Postoperative incisional pain is classified as acute spontaneous pain. Sensitized and spontaneously activated peripheral sensory neurons mainly contribute to the initiation of postoperative incisional pain (33), while peripheral and central inflammatory

responses play a certain role in postoperative incisional pain. Systemic or intrathecal administration of non-steroidal anti-inflammatory drugs shows a weak analgesic effect on postoperative incisional pain (34). Furthermore, microglia and astrocytes in the spinal cord are rapidly and transiently activated after paw incision, which is accompanied with mechanical allodynia (35,36). Although we cannot fully explain why postoperative incisional pain was not affected in TRPM2-KO mice, it may include a strong aspect of nociceptive pain, rather than inflammatory pain. Alternatively, postoperative incisional pain is caused by an incision of skin and deep tissue including fascia and muscle layer, resulting in the enhanced activity of muscle-innervating primary sensory neurons and the dorsal horn neurons received from muscle (37). Muscle pain induced by deep tissue incision may be less affected by TRPM2-mediated inflammation. The present results indicate that the role of TRPM2 is minimal in postoperative incisional pain, although further investigations will be needed to elucidate this discrepancy.

Osteoarthritis, the most common joint disease, is characterized by the degeneration of articular cartilage, and the major clinical symptoms are chronic pain and disability. Osteoarthritis is not considered as a classical inflammatory arthropathy, because of no manifestations of inflammation and absence of neutrophils in the synovial fluid. However, dysfunction of the chondrocytes is caused by synovial inflammation, including the infiltration of activated B-cells and T-lymphocytes and the production of proinflammatory mediators (38). Thus, osteoarthritis pain is induced by not only local tissue damage, but also these complex causes, which lead to peripheral sensitization (39). Furthermore, the activation of microglia has been observed, and a microglial inhibitor, minocycline, attenuated mechanical allodynia in MIA-induced osteoarthritis pain model (40). Taken together, the present results suggest that TRPM2 may be involved in immune and inflammatory processes in the knee joint and/or activation of spinal microglia activation associated with osteoarthritis in MIA-induced osteoarthritis pain model.

EAE is an animal model of multiple sclerosis, an autoimmune disease of the central nervous system (CNS). The pathological characteristics of multiple sclerosis include the infiltration of T-cells into the CNS, demyelination, and axonal degeneration in the CNS (41). Consistent with the present results, Melzer *et al.* reported that TRPM2-KO mice exhibited attenuated EAE scores, and reduced inflammatory and demyelinating spinal cord lesions. Furthermore, they showed that TRPM2 plays a critical role in T-cell proliferation and the secretion of proinflammatory cytokines, such as IL-2, interferon- γ and IL-17, following polyclonal T-cell receptor stimulation (42). These findings suggest that the modulation of T-cell effectors via TRPM2 contribute to exhibit EAE symptoms. On the other hand, mice immunized with MOG show mechanical and cold allodynia, which are developed before the emergence of any neurological deficit signs, and is independent of the symptom severity (43). It is suggested that spinal infiltration of T-cells and activation of astrocyte and microglia/macrophages in the spinal cord may play a critical role in allodynia in EAE model (43–45). We recently reported that TRPM2 is involved in the spinal infiltration of peripheral immune cells in peripheral nerve injury-induced neuropathic pain model (16). Therefore, it is possible that the attenuation of mechanical allodynia in TRPM2-KO mice may be due to not only the inhibition of resident microglial activation, but also the inhibition of spinal infiltration of peripheral immune cells.

Paclitaxel exerts its anti-cancer effects by increasing the stability of tubulin polymers to inhibit cellular replication, while it frequently induces severe peripheral neuropathy. Although the mechanisms still remain unclear, paclitaxel-induced peripheral neuropathy is associated with the swollen and vacuolated

mitochondria in the peripheral sensory neurons (46). The mitochondrial dysfunction produces oxidative stress, which leads to neurotoxicity and axonal degeneration in peripheral sensory neurons. Accumulating evidence suggest that TRPM2 plays a critical role in oxidative stress-induced cell injury (9). Thus, it is suggested that TRPM2 contributes to paclitaxel-induced peripheral neuropathy via oxidative stress-induced injury of peripheral sensory neurons. In addition, the inflammatory responses of peripheral immune cells and spinal glial cells are also involved in chemotherapy-induced peripheral neuropathy (47,48), suggesting that peripheral and spinal neuroinflammation via TRPM2 contributes to the induction of peripheral and central sensitization associated with paclitaxel-induced peripheral neuropathy.

TRPM2 is involved in oxidative stress in the pancreatic β -cells (49), and the regulation of insulin secretion and glucose homeostasis in mice (50,51). In isolated β -cells prepared from TRPM2-KO mice, glucose-induced insulin secretion was blunted in a high-glucose environment (50). Uchida *et al.* reported that TRPM2-KO mice showed higher blood glucose levels than WT mice in intraperitoneal glucose tolerance tests (50), while Zhang *et al.* showed improvement of glucose tolerance in TRPM2-KO mice (51). The present results showed that basal fasting blood glucose levels did not differ between WT and TRPM2-KO mice. Painful neuropathy is one of the most common complications of diabetes. Hyperglycemia, dyslipidemia, and insulin resistance are responsible for diabetic neuropathy. Several lines of evidence suggest that inflammation and mitochondrial dysfunction resulting from the diabetic state contribute to oxidative stress and nerve injury in diabetic neuropathy (52,53). Thus, TRPM2 may contribute to STZ-induced painful diabetic neuropathy via the induction of peripheral sensitization by peripheral nerve inflammation and oxidative stress-induced nerve injury. Furthermore, the activation of spinal microglia is a crucial component of diabetic neuropathic pain (54). Therefore, it is also possible that the inhibition of activated microglia by TRPM2 deficiency decreases STZ-induced diabetic neuropathic pain.

5. Conclusion

The present results demonstrated that TRPM2 deficiency has anti-allodynic effects in a wide range of inflammatory and neuropathic pain mouse models, including acute inflammatory pain, osteoarthritis pain, chronic pain in multiple sclerosis, chemotherapy-induced peripheral neuropathy, and diabetic painful neuropathy, as well as in classical inflammatory pain and peripheral nerve injury-induced neuropathic pain, which are based on peripheral and central neuroinflammation, rather than nociceptive pain. These findings suggest that TRPM2 is a promising drug target for these pathological pains.

Conflicts of interest

The authors indicated no potential conflicts of interest.

Acknowledgments

The authors would like to thank Dr. Fumimasa Amaya (Department of Anesthesiology, Kyoto Prefectural University of Medicine, Kyoto, Japan) for teaching us the techniques used in studying the postoperative incisional pain model. This work was supported in part by Grants-in-Aids for Scientific Research from the Ministry of Education, Culture, Sport, Science and Technology of Japan (a Grant-in-Aid for Scientific Research on Innovative Area 'Brain Environment' to T.N. [24111527]), and the Japan Society for the Promotion of Science (Grant-in-Aids for Young Scientist (B) to T.N.

[23790641] and Scientific Research (B) to S.K. [24390016] and T.N. [26293019]), and by a grant from the Salt Science Research Foundation, No. 14C4.

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